End of Result Set

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L1: Entry 1 of 1 File: USPT

DOCUMENT-IDENTIFIER: US 6379964 B1
TITLE: Evolution of whole cells and organisms by recursive sequence recombination

Detailed Description Text (293):
Many strains of S. cerevisiae have been isolated from high-ethanol environments, and have survived in the ethanol-rich environment by adaptive evolution. For example, strains from Sherry wine aging ("Flor" strains) have evolved highly functional mitochondria to enable their survival in a high-ethanol environment. It has been shown that transfer of these wine yeast mitochondria to other strains increases the recipient's resistance to high ethanol concentration, as well as thermotolerance (Jimenez, J. and Benitez, T (1988) Curr. Genet. 13: 461-469). There are several flor strains deposited in the ATCC, for example S. cerevisiae MY91 (ATCC 201301), MY138 (ATCC 201302), C5 (ATCC 201298), ET7 (ATCC 201299), LA6 (ATCC 201300), OSB21 (ATCC 201303), F23 (S. globosus ATCC 90920). Also, several flor strains of S. uvarum and Torulaspora prelorieisis have been deposited. Other ethanol-tolerant wine strains include S. cerevisiae ACA 174 (ATCC 60868), 15% ethanol, and S. cerevisiae A54 (ATCC 90921), isolated from wine containing 18% (v/v) ethanol, and NRCC 202036 (ATCC 46534), also a wine yeast. Other S. cerevisiae ethanologens that additionally exhibit enhanced ethanol tolerance include ATCC 24858, ATCC 24858, G 3706 (ATCC 42594), NRRL Y-265 (ATCC 60593), and ATCC 24845-ATCC 24860. A strain of S. pastorianus (S. carlsbergensis ATCC 2345) has high ethanol-tolerance (13% v/v). S. cerevisiae Sa28 (ATCC 26603), from Jamaican cane juice sample, produces high levels of alcohol from molasses, is sugar tolerant, and produces ethanol from wood acid hydrolyzate.

Detailed Description Text (306):
In one aspect, organisms having increased ethanol tolerance are selected for. A population of natural S. cerevisae isolates are mutagenized. This population is then grown under fermentor conditions under low initial ethanol concentrations. Once the culture has reached saturation, the culture is diluted into fresh medium having a slightly higher ethanol content. This process of successive dilution into medium of incrementally increasing ethanol concentration is continued until a threshold of ethanol tolerance is reached. The surviving mutant population having the highest ethanol tolerance are then pooled and their genomes recombined by any method noted herein. Enrichment could also be achieved by a continuous culture in a chemostat or turbidostat in which temperature or ethanol concentrations are progressively elevated. The resulting shuffled population are then exposed once again to the enrichment strategy but at a higher starting medium ethanol concentration. This strategy is optionally applied for the enrichment of thermotolerant cells and for the enrichment of cells having combined thermo- and ethanol tolerance.

End of Result Set

☐ Generate Collection

L1: Entry 1 of 1 File: USPT Apr 30, 2002

US-PAT-NO: 6379964
DOCUMENT-IDENTIFIER: US 6379964 B1

TITLE: Evolution of whole cells and organisms by recursive sequence recombination

DATE-ISSUED: April 30, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
del Cardayre; Stephen	Belmont	CA		
Tobin; Matthew	San Jose	CA		
Stemmer; Willem P. C.	Los Gatos	CA		
Minshull; Jeremy	Menlo Park	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Maxygen, Inc.	Redwood City	CA			02

APPL-NO: 09/ 354922 [PALM]
DATE FILED: July 15, 1999

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATION This application is a continuation-in part of U.S. Ser. No. 09/116,188, filed Jul. 15, 1998, which claims priority to U.S. Ser. No. 60/035,054, filed Jan. 17, 1997, and PCT/US/98/00852, filed Jan. 16, 1998 (designating the U.S.). The subject application claims priority to each of these prior applications, each of which is also incorporated by reference in its entirety for all purposes.

INT-CL: [07] C12 N 15/00, C07 H 21/02, C07 H 21/04

US-CL-ISSUED: 435/440; 536/23.1, 536/24.3, 935/76, 935/77, 935/78
US-CL-CURRENT: 435/440; 536/23.1, 536/24.3

FIELD-OF-SEARCH: 435/440, 435/6, 435/91.2, 536/23.1, 536/24.3, 935/76, 935/77, 935/78

PRIOR-ART-DISCLOSED:

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Search Selected | Search ALL

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☐	4729951	March 1988	Ferenczy et al.	
☐	4940834	July 1990	Hurley et al.	
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☐	5512463	April 1996	Stemmer
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☐	5929250	July 1999	Short
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☐	5939300	August 1999	Robertson et al.
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ART-UNIT: 1655

PRIMARY-EXAMINER: Whisenant; Ethan

ABSTRACT:

The invention provides methods employing iterative cycles of recombination and selection/screening for evolution of whole cells and organisms toward acquisition of desired properties. Examples of such properties include enhanced recombinogenicity, genome copy number, and capacity for expression and/or secretion of proteins and secondary metabolites.

23 Claims, 38 Drawing figures

End of Result Set

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L3: Entry 1 of 1 File: USPT

DOCUMENT-IDENTIFIER: US 6379964 B1
TITLE: Evolution of whole cells and organisms by recursive sequence recombination

Brief Summary Text (27):
The next step is to select or screen to isolate regenerated cells that have evolved toward acquisition of the desired property. DNA exchange and selection/screening steps are repeated, as needed, with regenerated cells in one cycle being used to form protoplasts in the next cycle until the regenerated cells have acquired the desired property. Industrial microorganisms are a preferred class of organisms for conducting the above methods. Some methods further comprise a step of selecting or screening for fused protoplasts free from unfused protoplasts of parental cells. Some methods further comprise a step of selecting or screening for fused protoplasts with hybrid genomes free from cells with parental genomes. In some methods, protoplasts are provided by treating individual cells, mycelia or spores with an enzyme that degrades cell walls. In some methods, the strain is a mutant that is lacking capacity for intact cell wall synthesis, and protoplasts form spontaneously. In some methods, protoplasts are formed by treating growing cells with an inhibitor of cell wall formation to generate protoplasts.

Detailed Description Text (246):
Another goal of shuffling yeast is to increase the tolerance of yeast to ethanol. Such is useful both for the commercial production of ethanol, and for the production of more alcoholic beers and wines. The yeast strain to be shuffled acquires genetic material by exchange or transformation with other strain(s) of yeast, which may or may not be know to have superior resistance to ethanol. The strain to be evolved is shuffled and shufflants are selected for capacity to survive exposure to ethanol. Increasing concentrations of ethanol can be used in successive rounds of shuffling. The same principles can be used to shuffle baking yeasts for improved osmotolerance.

Detailed Description Text (290):
S. cerievisiae is highly amenable to development for optimized SSF processes. It inherently possesses several traits for this use, including the ability to import and ferment a variety of sugars such as sucrose, glucose, galactose, maltose and maltriose. Also, yeast has the capability to flocculate, enabling recovery of the yeast biomass at the end of a fermentation cycle, and allowing its re-use in subsequent bioprocesses. This is an important property in that it optimizes the use of nutrients in the growth medium. S. cerevisiae is also highly amenable to laboratory manipulation, has highly characterized genetics and possesses a sexual reproductive cycle. S. cerevisiae may be grown under either aerobic or anaerobic conditions, in contrast to some other potential SSF organisms that are strict anaerobes (e.g. (Clostridium spp.), making them very difficult to handle in the laboratory. S. cerevisiae are also "generally regarded as safe" ("GRAS"), and, due to its widespread use for the production of important comestibles for the general public (e.g. beer, wine, bread, etc), is generally familiar and well known. S. cerevisiae is commonly used in fermentative processes, and the familiarity in its handling by fermentation experts eases the introduction of novel improved yeast strains into the industrial setting.

Detailed Description Text (306):
In one aspect, organisms having increased ethanol tolerance are selected for. A population of natural S. cerevisae isolates are mutagenized. This population is then grown under fermentor conditions under low initial ethanol concentrations. Once the culture has reached saturation, the culture is diluted into fresh medium having a slightly higher ethanol content. This process of successive dilution into medium of

incrementally increasing ethanol concentration is continued until a threshold of ethanol tolerance is reached. The surviving mutant population having the highest ethanol tolerance are then pooled and their genomes recombined by any method noted herein. Enrichment could also be achieved by a continuous culture in a chemostat or turbidostat in which temperature or ethanol concentrations are progressively elevated. The resulting shuffled population are then exposed once again to the enrichment strategy but at a higher starting medium ethanol concentration. This strategy is optionally applied for the enrichment of thermotolerant cells and for the enrichment of cells having combined thermo- and ethanol tolerance.

Detailed Description Text (519):
Approximately 10.sup.9 S. coelicolor spores were inoculated into 50 ml YEME with 0.5% Glycine in a 250 ml baffled flask. The spores were incubated at 30.degree. C. for 36-40 hours in an orbital shaker. Mycelium were verified using a microscope. Some strains needed an additional day of growth. The culture was transferred into a 50 ml tube and centrifuged at 4,000 rpm for 10 min. The mycelium were twice washed with 10.3% sucrose and centrifuged at 4,000 rpm for 10 min. (mycelium can be stored at -80.degree. C. after wash). 5 ml of lysozyme was added to the .about.0.5 g of mycelium pellet. The pellet was suspended and incubated at 30.degree. C. for 20-60 min., with gentle shaking every 10 min. The microscope was checked for protoplasting every 20 min. Once the majority were protoplasts, protoplasting was stopped by adding 10 ml of P buffer. The protoplasts were filtered through cotton and the protoplast spun down at 3,000 rpm for 7 min at room temperature. The supernatant was discarded and the protoplast gently resuspended, adding a suitable amount of P buffer according to the pellet size (usually about 500 .mu.l). Ten-fold serial dilutions were made in P buffer, and the protoplasts counted at a 10.sup.-2 dilution. Protoplasts were adjusted to 10.sup.10 protoplasts per ml.

Detailed Description Text (560):
To assay, 50 .mu.L of supernatant is added to 100 .mu.L of distilled water in a 96 well polypropylene microtitre plate, and the plate is centrifuged at 4000 rpm to pellet the mycelia. 50 .mu.L of the cleared supernatant is then removed and added to a flat bottom polystyrene 96 well microtitre plate containing 150 .mu.L 1M KOH in each well. The resulting plates are then read in a microtitre plate reader measuring the absorbance at 654 nm of the individual samples as a measure of the content .gamma.-actinorhodin.

Detailed Description Text (567):
Approximately 10.sup.9 protoplasts were centrifuged at 3,000 rpm for 7 min. When more than one strain was used, equal number of protoplasts were obtained from each strain. Most of the buffer was removed and the pellet suspended in the remaining buffer (.about.25 .mu.l total volume) by gentle flicking. 0.5 ml of 50% PEG1000 was added and mixed with the protoplasts by gently pipetting in and out 2 times. The mixture was then incubated for 2 minutes. 0.5 ml of P buffer was added and gently mixed. (This is the fusion at a dilution of 10.sup.-1). A ten-fold serial dilution was performed in P buffer. After 2 minutes, dilutions were plated at 10.sup.-1, 10.sup.-2 and 10.sup.-3 onto R5 plates with 50 .mu.l of each, 2.sup.-3 plates each dilution. (for plating, -20 of 3 mm glass beads were used, gentle shaking). As a first control, for regeneration of protoplasts, the same number of protoplasts were used as above, adding P buffer to a total of 1 ml (this is the regeneration at dilution 10.sup.-1). The mixture was further diluted (10.times.) in P buffer. The dilutions were plated at 10.sup.-3, 10.sup.-4 and 10.sup.-5 onto R5 plates with 50 .mu.l of each. As a second control, (as a non-protoplasting mycelia background check) the same number of protoplasts as above were used adding 0.1% SDS to a total of 1 ml (this is the background at dilution 10.sup.-1). After further 10.times. dilution in 0.1% SDS, the dilution was plated at 10.sup.-1, 10.sup.-2 and 10.sup.-3 onto R5 plates with 50 .mu.l of each. The plates were air dried and Incubated at 30.degree. C. for 3 days.

CLAIMS:

8. The method of claim 1, further comprising selecting or screening to isolate regenerated cells with hybrid genomes free from cells with parental genomes.

END OF SEARCH HISTORY

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>>>  Use USPATALL when searching terms such as patent assignees,  <<<
>>>  classifications, or claims, that may potentially change from <<<
>>>  the earliest to the latest publication.                     <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> s l1
          299 CEREVISAE
       490757 DENSITY
       110596 TOLERANCE
       111347 PELLET?
        11404 BEER
       216289 ETHANOL
       953005 DIAMETER
      1092555 FREE
L2            1 CEREVISAE AND DENSITY AND TOLERANCE AND PELLET? AND BEER AND
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L2   ANSWER 1 OF 1  USPATFULL
TI    Evolution of whole cells and organisms by recursive sequence
      recombination
AN    2002:95605  USPATFULL
TI    Evolution of whole cells and organisms by recursive sequence
      recombination
IN    del Cardayre, Stephen, Belmont, CA, United States
      Tobin, Matthew, San Jose, CA, United States
      Stemmer, Willem P. C., Los Gatos, CA, United States
      Minshull, Jeremy, Menlo Park, CA, United States
PA    Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)
PI    US 6379964          B1   20020430
AI    US 1999-354922           19990715 (9)
RLI   Continuation-in-part of Ser. No. US 116188
PRAI  US 1997-35054P      19970117 (60)
DT    Utility
FS    GRANTED
EXNAM Primary Examiner: Whisenant, Ethan
LREP  Kruse, Norman J., Quine, Jonathon Alan, The Law Offices of Jonathan Alan
      Quine
CLMN  Number of Claims: 23
ECL   Exemplary Claim: 1
DRWN  38 Drawing Figure(s); 41 Drawing Page(s)
LN.CNT 7147
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB    The invention provides methods employing iterative cycles of
      recombination and selection/screening for evolution of whole cells and
      organisms toward acquisition of desired properties. Examples of such
      properties include enhanced recombinogenicity, genome copy number, and
      capacity for expression and/or secretion of proteins and secondary
      metabolites.


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WEST Search History

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L5	l4 and pellet$	29	L5
L4	L2 and density	45	L4
L3	L2 and floc	1	L3
L2	saccharomyces cerevisae and ethanol	103	L2
L1	saccharomyces cerevisae and ethanol and beer	7	L1

END OF SEARCH HISTORY